REMARKS

The Pending Claims:

Before entry of the foregoing amendments,Claims 1-62, 75-82, 94-101, and 107-117, are pending in this application. Claims 1-13, 22-34, 43-53, and 83-93 are directed to a method of obtaining a selectable transgenic stem cell of a vertebrate. Claims 14-17, 35-38, 54-57, 75-78, 94-97, are drawn to a selectable transgenic stem cell, and Claims 18-19, 39-40, 58-59, 79-80, and 98-99 relate to a transgenic non-human vertebrate comprising the stem cell. Claim 60 relates to a maturing male gamete obtained by the method. Claims 20, 41, 61, 81, and 100 relate to vertebrate semen comprising a maturing male gamete obtained by the method. Claims 21, 42, 62, 82, and 101 relate to a method of producing a non-human transgenic vertebrate animal line. Claims 107 and 110-114 relate to a transgenic vertebrate cell containing the nucleic acid construct, and Claims 108-109 and 115-117 relate to a transgenic non-human vertebrate comprising the cell.

The Office Action and Applicant's Amendment

Applicant's Amendment, filed February 9, 2001, was acknowledged.

Applicant's election of Group I and species "mammal" were acknowledged.

The cancellation of Claims 63-74, 83-93, 102-106, and 118-132, without prejudice, and the amendment of Claims 1, 75, 94, and 107, were acknowledged.

Claims 1-62, 75-82, 94-101, and 107-117, were objected to because the claimed invention "has not been amended to read only on the elected invention." The Examiner required appropriate correction. Applicant has canceled Claims 1-62, 75-82, 94-101, and 107-117, mooting the objection. Applicant's new Claims 133-195, correspond to elected claim Group I and lack any recitation of embodiments related to non-mammalian vertebrates, e.g., birds, and other non-elected species.

In the present amendment Applicant has canceled Claims 1-62, 75-82, 94-101, and

107-117, without prejudice, and has added new Claims 133-195.

Support for Claim 133 is found, for example, in Claims 22 and 8 as originally filed, and in the specification, at page 14, lines 16-23; and at page 15, lines 4-28.

Support for Claim 134 is found, for example, in Claim 23 as originally filed.

Support for Claim 135 is found, for example, in Claim 24 as originally filed.

Support for Claim 136 is found, for example, in Claims 25 as originally filed.

Support for Claims 137 is found, for example, in Claim 26 as originally filed.

Support for Claim 138 is found, for example, in Claim 27 as originally filed.

Support for Claim 139 is found, for example, in Claim 28 as originally filed.

Support for Claim 140 is found, for example, in Claim 30 as originally filed.

Support for Claim 141 is found, for example, in Claim 32 as originally filed.

Support for Claim 142 is found, for example, in Claim 33 as originally filed.

Support for Claim 143 is found, for example, in Claim 34 as originally filed.

Support for Claim 144 is found, for example, in Claim 35 as originally filed.

Support for Claim 145 is found, for example, in Claim 38 as originally filed.

Support for Claim 146 is found, for example, in Claim 39 as originally filed.

Support for Claim 147 is found, for example, in Claim 41 as originally filed.

Support for Claim 148 is found, for example, in Claim 42 as originally filed.

Support for Claim 149 is found, for example, in Claim 43 and Claim 45 as originally filed, and in the specification, e.g., at page 14, lines 16-23; and at page 15, lines 4-28.

Support for Claim 150 is found, for example, in Claim 44 as originally filed.

Support for Claim 151 is found, for example, in Claim 46 as originally filed.

Support for Claim 152 is found, for example, in Claim 47 as originally filed.

Support for Claim 153 is found, for example, in Claim 48 as originally filed.

Support for Claim 154 is found, for example, in Claim 50 as originally filed.

Support for Claim 155 is found, for example, in Claim 52 as originally filed.

Support for Claim 156 is found, for example, in Claim 53 as originally filed.
Support for Claim 157 is found, for example, in Claim 54 as originally filed.
Support for Claim 158 is found, for example, in Claims 55 as originally filed.
Support for Claims 159 is found, for example, in Claim 56 as originally filed.
Support for Claim 160 is found, for example, in Claim 57 as originally filed.
Support for Claim 161 is found, for example, in Claim 58 as originally filed.
Support for Claim 162 is found, for example, in Claim 60 as originally filed.
Support for Claim 163 is found, for example, in Claim 61 as originally filed.
Support for Claim 164 is found, for example, in Claim 62 as originally filed.
Support for Claim 165 is found, for example, in Claim 75 as originally filed.
Support for Claim 166 is found, for example, in Claim 76 as originally filed.
Support for Claim 167 is found, for example, in Claim 77 as originally filed.
Support for Claim 168 is found, for example, in Claim 78 as originally filed.
Support for Claim 169 is found, for example, in Claim 79 as originally filed.
Support for Claim 170 is found, for example, in Claim 81 as originally filed.
Support for Claim 171 is found, for example, in Claim 82 as originally filed.
Support for Claim 172 is found, for example, in Claim 107 as originally filed.
Support for Claim 173 is found, for example, in Claim 108 as originally filed.
Support for Claim 174 is found, for example, in Claim 110 as originally filed.
Support for Claim 175 is found, for example, in Claim 111 as originally filed.
Support for Claim 176 is found, for example, in Claim 112 as originally filed.
Support for Claim 177 is found, for example, in Claim 113 as originally filed.
Support for Claims 178 is found, for example, in Claim 114 as originally filed.
Support for Claim 179 is found, for example, in Claims 115 as originally filed.
Support for Claim 180 is found, for example, in Claim 116 as originally filed.
Support for Claim 181 is found, for example, in Claims 22 and 30 as originally filed, and in the specification, at page 14, lines 16-23; and at page 15, lines 4-28.

Support for Claim 182 is found, for example, in Claim 23 as originally filed.
Support for Claim 183 is found, for example, in Claim 24 as originally filed.
Support for Claim 184 is found, for example, in Claims 25 as originally filed.
Support for Claim 185 is found, for example, in Claim 26 as originally filed.
Support for Claim 186 is found, for example, in Claim 27 as originally filed.
Support for Claim 187 is found, for example, in Claim 28 as originally filed.
Support for Claim 188 is found, for example, in Claim 32 as originally filed.
Support for Claim 189 is found, for example, in Claim 33 as originally filed.
Support for Claim 190 is found, for example, in Claim 34 as originally filed.
Support for Claim 191 is found, for example, in Claim 35 as originally filed.
Support for Claim 192 is found, for example, in Claim 38 as originally filed.
Support for Claim 193 is found, for example, in Claim 39 as originally filed.
Support for Claim 194 is found, for example, in Claim 41 as originally filed.
Support for Claim 195 is found, for example, in Claim 42 as originally filed.

The Examiner stated that the claims are free of the prior art of record, because the prior art fails to teach techniques for transfecting germ cells via administration of a stem cell-specific promoter construct to gonad.

The Examiner acknowledged the claimed methods, cells, and mammals are enabled for transgenic mice comprising the human cyclin A1 promoter construct.

However, no claims (pending before the present amendment) were allowed, and rejections were based on the following grounds.

I. Rejection based on Obviousness-type Double Patenting

Claims 1-62, 75-82, 94-101, and 107-117 were provisionally rejected based on obviousness-type double patenting over Claims 135-149, 151, 157-163, 171, and 199 of copending U.S. Serial No. 09/191,920, and over Claims 124-139 and 157-159 of copending U.S. Serial No. 09/272,443.

Applicant has herein canceled Claims 1-62, 75-82, 94-101, and 107-117, with respect to which the rejection is mooted.

With respect to new Claims 133-195, Applicant herewith submits a terminal disclaimer as to U.S. Serial No. 09/191,920, for which Applicant has already paid the issue fee. Therefore, Applicant respectfully requests the Examiner to withdraw the provisional rejection with respect to U.S. Serial No. 09/191,920.

At such time as there is a finding of allowable subject matter in U.S. Serial No. 09/272,443, Applicant will consider executing a terminal disclaimer as to the allowable subject matter in the present application.

II. Rejections based on 35 U.S.C. § 112, first paragraph

Claim 1-62, 75-82, 94-101, and 107-117 were rejected under 35 U.S.C. 112, first paragraph, based on the following:

> **Claims 1-62, 75-82, 94-101, and 107-117 are rejected under 35 U.S.C. 112, first paragraph, while being enabling for methods for obtaining selectable transgenic stem cells from transgenic phCyclinAl-EGFP mice, does not reasonably provide enablement for methods and products encompassing any and all transgenic mammals comprising any and all stem cell-specific promoters operably linked to DNA encoding fluorescent or light-emitting protein. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention commensurate in scope with these claims.**
>
> **The specification does not enable one of skill in the art to make or use the claimed invention. Each of the claims require transfection of male mammalian germ cells via administration to the gonad (as per the election of Paper No. 11).**
>
> **The specification discloses that the invention arose from a desire to improve the production of transgenic animals. It is noted that the claims include the genetic modification of human germ cells for transfer into human or non-human germ cells. As the specification fails to teach how to make or use such genetically modified human germ cells, it is suggested that the claims be limited to "non-human" because germline gene therapy is an unpredictable and undeveloped art on the basis that RAC of NIH will not consider any germ-line gene therapy protocols, and that people skilled in the art consider that the technology for human germ-line manipulation is too inefficient and too preliminary in its development for consideration. See page 79 of Gene Therapy, A Handbook for Physicians. Although the specification indicates that the method could be used potentially to correct genetic defects which cause infertility, the specification teaches no genetic sequence which functions to correct any fertility problem. In fact, no gene is known to date that would correct a fertility problem, much less be delivered via germline gene transfer.**
>
> **The state of the art of making transgenic mammals is fairly established in regards to the methods where fertilized oocytes are microinjected or in mice where ES cells are used. The presently claimed method, wherein the sperm cells are transfected (via administration to a gonad) is not well-established and is unpredictable. The method has been tried in the past with varying degrees of success. Sato et al. (IDS) report that the system was unsuccessful in producing transgenic offspring. Lavitrano et al. (IDS) reported success in making transgenic offspring, but, as reported by Brinster et al. (No simple solution...) (IDS), the results were not repeatable. Later, in 1999, Chang et al. (IDS) report success in making transgenic rats using liposome-complexed DNA. It is not clear why the earlier reports were not successful in making transgenic offspring. Sato et al. speculate that the mode of transfection was the reason that their method was not successful. Applicant's preferred and exemplified method of transfection uses a stem cell-specific promoter construct, particularly human cyclin A1 promoter. Accordingly, in view of the unpredictable state of the art, the claimed methods, cells, and mammals are enabled only for the transgenic mice**

comprising the human cyclin A1 promoter construct . . .

. . . Furthermore, the claims require the use of a stem-cell specific promoter, particularly human cyclin Al promoter. The specification fails to teach that stem-cell specific transgene expression using any other promoter construct. It is well known in the art that the state of the transgenic art is unpredictable, particularly at the time of effective filing, even within the mouse system, with regard to transgene behavior and the resulting phenotypic effect. See Wall (Theriogenology, 1996) who reports that "our lack of understanding of genetic control elements makes it difficult to design transgenes with predictable behavior." See page 61, last paragraph.

In conclusion, in view of the nature of the invention, the state of the prior art, the lack of predictability found in the art, the breadth of the claims, the lack of appropriate guidance, and the lack of correlatable working examples, it would require undue experimentation to make or use the instant invention encompassing any and all transgenic mammals having any and all stem cell specific promoters.

Applicant has herein canceled Claims 1-62, 75-82, 94-101, and 107-117, with respect to which the rejection is mooted.

Applicant's new Claims 133-195 are directed to transfection employing a "polynucleotide comprising a transcriptional unit of a human cyclin A1 promoter sequence operatively linked to a DNA encoding a fluorescent or light-emitting protein." (E.g., Claim 133, 149, 165, and 181). Therefore, the Examiner's comments concerning "any and all stem cell-specific promoters" do not apply to new Claims 133-195.

With respect to new Claims 181-195, the Examiner's comments concerning "any and all transgenic [non-human] mammals" do not apply, because these claims are directed to a "method of obtaining a selectable transgenic stem cell of a mouse" (e.g., Claim 181 and claims dependent therefrom), "a selectable transgenic stem cell obtained by the method of Claim 181" (e.g., Claim 191), "a transgenic mouse comprising the selectable transgenic stem cell of Claim 191," "semen of a male mouse comprising a male gamete obtained by the method of claim 188" (e.g., Claim 194), and "a method of producing a transgenic murine line having native germ cells" (e.g., Claim 195).

With respect to new Claims 133-180, Applicant asserts that these claims are enabled as to non-human mammals, in general, and not only as to mice, which are useful models for gene expression and transgenesis in mammals generally. The Examiner has noted that the Wall reference states that while ". . . transgene expression and the physiological consequences of transgene products in livestock are not *always* accurately predicted in transgenic mouse studies" and "[c]urrently, the only approach that yields truly informative

data is testing transgenes in the livestock species of interest." (Wall, at page 62; emphasis added). Neveretheless, the Examiner appears to have ignored that Wall also states that testing in the species of interest ". . . is obviously an unsatisfactory, time-consuming, expensive testing option" and that ". . . a reasonable amount of useful information about transgene function *can be* derived from transgenic mouse studies." (Wall at page 62, last paragraph, immediately following and preceding the statement highlighted by the Examiner).

In addition, the claimed method involves selection and isolation of transfected stem cells, and useful phenotypic screening means for the effectiveness of a given nucleic acid construct in particular mammals of interest are known and predictable, as taught, for example, by the cited Wall reference (e.g., at page 62, last eight lines of the last paragraph).

Moreover, the usefulness of murine models for genetic expression and transgenesis in other mammals is widely accepted. This is demonstrated by the fact that the National Institutes of Health have devoted substantial resources to establishing a National Repository for Transgenic Mice and Rats at the Jackson Laboratory (see, **Exhibit B**: National Center for Research Resources [NCRR] website, excerpt). Abundant sources have reported the usefulness of murine models for studying genetic expression and transgenesis in other mammals. (E.g., **Exhibit C**: Moldin, SO *et al.*, *Trans-NIH neuroscience initiatives on mouse phenotyping and mutagenesis,* Mamm. Genome 12(8):575-81 [2001], Abstract; **Exhibit D**: Duff, K et al., Progress in the modeling of neurodegenerative diseases in transgenic mice, Curr. Opin. Neurol. 14(4):441-47 [2001], Abstract; **Exhibit E**: Duff, K., Transgenic mouse models of Alzheimer's disease: phenotype and mechanisms of pathogenesis, Biochem. Soc. Symp. 67:195-202 [2001], Abstract).

With respect to new Claims 133-195, Applicant agrees that the methods for transfecting male germ cells that have been used in the past have been largely unsuccessful. The reason for the lack of success has been due the methods used for transfection. For

instance Sato *et al.* (1994) used direct injection of foreign DNA into the mouse testis and were not successful in permanently transfecting testis cells. This was probably due to the calcium phosphate method of transfection that was used. This method has a relatively low transfection efficiency in vitro and is likely to be even lower in vivo. Lavitrano *et al.* immersed mature sperm in a DNA solution in order to transfect these cells. Many laboratories including Brinster's, have shown that Lavitrano's method does not lead to transfection of spermatozoa. Therefore, it is clear that the method of transfection employed is clearly important for success. But as the Declaration of Dr. Readhead (**Exhibit A**) describes, the transfection methods employed in accordance with the claimed methods, for genetic modification of male germ cells, were indeed successful in incorporating the polynucleotide into the genomes of the germ cells.

While new Claims 133-195 are not directed to germ line gene therapy in humans, it should be noted that, contrary to the Examiner's assertion, there are several genes that are known to be directly related to fertility, and which can be targeted by gene therapy. A mutation or targeted disruption of these genes results in infertility. For instance a mutation in the ataxia-telangiectasia (ATM) gene is known to cause infertility in humans. Similarly, targeted disruption of the ATM gene caused infertility in mice (Yu *et al.*, *Targeted distruption of ATM leads to growth retardation, chromosomal fragmentation during meiosis, immune defects and thymic lymphoma* Genes and Development 10:2411-2422 [1996]). Other examples of gene mutations that directly result in infertility are: c-kit (Geissler, E. *et al.*, *The dominant white spotting (W) locus of the mouse encodes the c-kit proto-oncogene*, Cell 55: 185-192 [1988]); cyclin A1 (Sweeney, C. et al., A distinct cyclin A is expressed in germ cells in the mouse. Development 122: 53-54 [1996]); and azoospermia factor (AZF; Mahadenvaiah, S.K. *et al.*, *Mouse homologues of the human AZF candidate gene RBM are expressed in spermatogonia and spermatids and map to a Y chromosome deletion interval associated with a high incidence of sperm abnormalities*, Human Molecular Genetics 7:715-727 [1998]).

The Examiner further stated:

> **. . . To this end, DNA associates tightly with spermatozoa such that demonstrations of presence of transfected cells in the testes or in ejaculate does not demonstrate that the germ cells were actually transfected. As such, it is not clear as to whether the sperm cells are more or less susceptible to transfection at particular stages. Because it is not known as to which, if any, types of sperm forming cells are transfected it is not clear as to how long transfected germ cells would remain in a particular animal. These parameters appear to be very important because the timing of the matings may need to be controlled by the duration of the transfected sperm cells . . .**

Applicant strongly disagrees with the Examiner's assertion that Applicants have failed to demonstrate that the germ cells were actually transfected. The specification, e.g., in Example 7, shows the presence of GFP DNA (genetic marker gene) in the testis of experimental animals nine days after receiving the transferrin-polylysine adenoviral vector-containing transfection mixture. Further, Example 8 demonstrates the expression of GFP protein in the seminiferous tubules of male vertebrates. Together, these results show that the adenoviral vectors employed were able to tranfect male germ cells in vivo and that the germ cells expressed GF protein from the transgene at least nine days after introduction of the viral/DNA mixture into the testis. Contrary to Examiner's assertion that "DNA associates tightly with spermatozoa such that demonstrations of presence of transfected cells in the testes or in ejaculate does not demonstrate that the germ cells were actually transfected," DNA associated on the exterior surface of spermatozoa under normal circumstances would be degraded within 24 hours. Therefore, the disclosures of the specification that exogenous DNA remained and was expressed at least nine days after administration of the transfection mixture, indicates that the exogenous DNA was either found episomally in the nucleus or integrated into the chromosomes. Since the overwhelming majority of cells in the seminiferous tubules are male germ cells at various stages of maturation, the observation of fluorescence in the seminiferous tubules, provided in Example 8, strongly demonstrates that male germ cells were indeed transfected, at least transiently. Examples 19, 20, and 21further demonstrate that transgenic mammals were able to express the xenogeneic fluorescent marker under the regulation of the human cyclin

A1 promoter.

Applicant further submits the Declaration of Dr. Carol W. Readhead (appended as **Exhibit A**), which demonstrates that exogenous DNA was successfully introduced into male germ cells, using a lentiviral vector, and that transgenic offspring were produced after natural mating.

The examiner stated that the specification failed to clarify at which developmental stage(s) the male germ cell is most receptive to transfection, and she suggested that this is crucial information for the successful timing of matings. Applicant agrees that it is unknown whether certain types of sperm-forming cells are more susceptible to transfection than others. However, the specification describes the developmental stages of male germ cells (e.g., page 3, line 15-28; page 14, lines 11-26) and that, in accordance with the claimed method, the exogenous nucleic acid segment will reach germ cells at one or more of these stages, to be taken up by those that are at a more receptive stage (page 14, lines 21-23). Therefore, no selection of the most receptive stage is necessary in the in vivo method.

Further, as long as fertile females are plentiful, the timing of matings is trivial to the skilled artisan. Spermatogenesis in vertebrates typically occurs within a turnover window of several days or weeks. For example, in the mouse, spermatogenesis takes 60 days from from spermatogonia to mature spermatozoon (Russell, L.D., *et al.* In: *Histological and Histopathological evaluation of the testis*, Cache River Press [1990]). If germ cell stem-cells are stably transfected then these transfected stem cells would constantly be replenishing the sperm cells and the male would be able to produce transgenic offspring throughout the reproductive life of the animal. If later cell stages were to be transfected then the male would be able to sire transgenic offspring for a period somewhat less than the typical turnover period, but a population of mature genetically modified sperm cells would be present for at least a period of days, during which numerous offspring could be generated. With the presence of a genetic marker (e.g., fluorescent protein), it is trivial to determine whether transgenic sperm are present in any given semen sample before using it

for breeding purposes.

In addition, due to germ line transmission of the integrated DNA, the transgenic offspring would then form founder animals capable of generating transgenic offspring of their own. Their ability to do so would last throughout the reproductive life of the animal. Therefore even if the transmission of the gene is limited to a narrow time window after administering the transfection mixture, the claimed method will still result in transgenic offspring that could then be used to further transmit the transgene.

Therefore, the Examiner is respectfully requested to withdraw the rejection on this ground.

III. Rejections based on 35 U.S.C. § 112, second paragraph

Claims 1-62, 75-82, 94-101, and 107-117 were rejected under 35 U.S.C. 112, second paragraph. The Examiner stated:

> **In the claims, the limitations "germ cell" or "maturing germ cell" are vague and indefinite as to what is intended to be encompassed within the metes and bounds of the claims. In particular, it appears that the specification teaches transfection of germ cells within the testis or gonad and fails to define what is meant by a "mature" sperm cell. Clarification and/or amendment to the claims is requested.**
>
> **In the claims, the limitation "at about or below the vertebrate's body temperature and for a transfection-effective period of time" is vague and indefinite with regard to what is intended to be claimed. In particular, it is unclear as to what the limitation pertains or to what the limitation modifies, i.e., gene delivery mixture, transfecting agent, transfecting, etc. Clarification and/or amendment to the claims is requested.**

As to canceled Claims 1-62, 75-82, 94-101, and 107-117, the grounds of rejection are moot.

With respect to new Claims 133-195, Applicant believes that the scope of "male germ cells" is clearly set forth. For example new Claims 133, 149, 165, and 181 recite that the male germ cell ". . . is selected from the group consisting of spermatogonial stem cells, type B spermatogonia, primary spermatocytes, preleptotene spermatocytes, leptotene spermatocytes, zygotene spermatocytes, pachytene spermatocytes, secondary spermatocytes, spermatids, and spermatozoa . . ."

In addition, none of new Claims 133-195 recites the word "maturing," with respect to male germ cells or male gametes. Applicant's intention in originally using the word "maturing" was merely the recognition that the development of male germ cells is an ongoing process and that the "final changes in the spermatozoon take place in the genital tract of the female, prior to fertilization." (Specification, at page 3, lines 27-28).

Similarly, none of new Claims 133-195 recites the phrase "at about or below the vertebrate's body temperature and for a transfection-effective period of time."

Consequently, Applicant respectfully requests the Examiner to withdraw the rejection on this ground.

CONCLUSION

In view of the above amendments and remarks, it is submitted that this application is now ready for allowance. If, in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject application, the Examiner is invited to call the undersigned attorney at (213) 896-6665.

Respectfully submitted,
SIDLEY & AUSTIN

By: _____

Nisan A. Steinberg, Ph.D.
Reg. No. 40,345

Sidley Austin Brown & Wood
555 West Fifth Street, Suite 4000
Los Angeles, CA 90013

VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Claims:

Please cancel Claims 1-62, 75-82, 94-101, and 107-117, without prejudice and add the following new Claims 133-195.

--133.(New) A method of obtaining a selectable transgenic stem cell of a non-human mammal, comprising:

injecting into a gonad of a male non-human mammal a transfection mixture comprising at least one transfecting agent and at least one polynucleotide comprising a transcriptional unit of a human cyclin A1 promoter sequence operatively linked to a DNA encoding a fluorescent or light-emitting protein, wherein said gonad contains a male germ cell of the non-human mammal, and wherein said germ cell is selected from the group consisting of spermatogonial stem cells, type B spermatogonia, primary spermatocytes, preleptotene spermatocytes, leptotene spermatocytes, zygotene spermatocytes, pachytene spermatocytes, secondary spermatocytes, spermatids, and spermatozoa;

causing said polynucleotide to be taken up by, and released into, said germ cell; and

incorporating said polynucleotide into the genome of said germ cell, whereby a selectable transgenic stem cell is obtained expressing said fluorescent or light-emitting protein, by which said stem cell can be isolated or selected from a non-stem cell.

134.(New) The method of Claim 133, further comprising, after incorporating said polynucleotide into the genome of said germ cell, breeding said male non-human mammal with a female of its species to obtain a transgenic progeny expressing said fluorescent or light-emitting protein in at least one of its stem cells.

135.(New) The method of Claim 134, wherein breeding is by in

vitro or in vivo fertilization of an ovum of said female.

136.(New) The method of Claim 133, wherein said cyclin A1 promoter sequence comprises SEQ. ID. NO.:2, or an operative fragment [or non-human homologue] thereof, or an operative derivative of any of these.

137.(New) The method of Claim 133, wherein said polynucleotide further comprises at least one insulator element flanking said transcriptional unit, whereby methylation in vivo of said promoter sequence is substantially prevented.

138.(New) The method of Claim 137, wherein at least one of said insulator element(s) is a chicken β-globin insulator element.

139.(New) The method of Claim 133, wherein said fluorescent or light-emitting protein is a green fluorescent protein, yellow fluorescent protein, blue fluorescent protein, phycobiliprotein, luciferase or apoaequorin.

140.(New) The method of Claim 133, wherein said non-human mammal is a non-human primate, a mouse, a rat, a rabbit, a gerbil, a hamster, a canine, a feline, an ovine, a bovine, a swine, a pachyderm, an equine, or a marine mammal.

141.(New) The method of Claim 133, wherein said germ cell develops into a male gamete after said polynucleotide is incorporated into the genome of said germ cell.

142.(New) The method of Claim 134, further comprising growing a stem cell of said progeny in vitro.

143.(New) The method of Claim 142, wherein said stem cell is grown in the presence of an inhibitor of DNA methylation.

144.(New) A selectable transgenic stem cell obtained by the method of Claim 133.

145.(New) The selectable transgenic stem cell of Claim 144, wherein said stem cell is a selectable transgenic male germ cell.

146.(New) A transgenic non-human mammal comprising the selectable transgenic stem cell of Claim 144.

147.(New) Semen of a non-human mammal comprising a male gamete obtained by the method of Claim 141.

148.(New) A method of producing a transgenic non-human mammalian line having native germ cells, comprising

breeding of the non-human mammal of Claim 146 with a member of the opposite sex of the same species; and selecting progeny for stem cell-specific expression of a xenogeneic fluorescent or light-emitting protein.

149.(New) A method of obtaining a selectable transgenic stem cell of a non-human mammal, comprising:

injecting into a gonad of a male non-human mammal a transfection mixture comprising at least one transfecting agent and at least one polynucleotide comprising a transcriptional unit of a cyclin A1 promoter sequence consisting of SEQ. ID. NO.:2, or an operative

fragment or derivative thereof, said promoter sequence operatively linked to a DNA encoding a fluorescent or light-emitting protein, wherein said gonad contains a male germ cell of the non-human mammal, and wherein said germ cell is selected from the group consisting of spermatogonial stem cells, type B spermatogonia, primary spermatocytes, preleptotene spermatocytes, leptotene spermatocytes, zygotene spermatocytes, pachytene spermatocytes, secondary spermatocytes, spermatids, and spermatozoa;

causing said polynucleotide to be taken up by, and released into, said male germ cell;

incorporating said polynucleotide into the genome of said germ cell;

allowing said male germ cell to develop into a male gamete; and

breeding said male non-human mammal with a female of its species to obtain a transgenic progeny expressing said fluorescent or light-emitting protein in at least one of its stem cells, whereby said stem cell can be isolated or selected from a non-stem cell.

150.(New) The method of Claim 149, wherein breeding is by in vitro or in vivo fertilization of an ovum of said female.

151.(New) The method of Claim 149, wherein said polynucleotide further comprises at least one insulator element flanking said transcriptional unit, whereby methylation in vivo of said promoter sequence is substantially prevented.

152.(New) The method of Claim 151, wherein at least one of said insulator element(s) is a chicken β-globin insulator element.

153.(New) The method of Claim 149, wherein said fluorescent or light-emitting protein is a green fluorescent protein, yellow fluorescent protein, blue fluorescent protein, phycobiliprotein, luciferase or apoaequorin.

154.(New) The method of Claim 149, wherein said non-human mammal is a non-human primate, a mouse, a rat, a rabbit, a gerbil, a hamster, a canine, a feline, an ovine, a bovine, a swine, a pachyderm, an equine, or a marine mammal.

155.(New) The method of Claim 149, further comprising growing a stem cell of said transgenic progeny in vitro.

156.(New) The method of Claim 155, wherein said stem cell is grown in the presence of an inhibitor of DNA methylation.

157.(New) A selectable transgenic stem cell obtained by the method of Claim 149.

158.(New) The selectable transgenic stem cell of Claim 157, wherein said stem cell is a pluripotent, multipotent, bipotent, or monopotent stem cell.

159.(New) The selectable transgenic stem cell of Claim 157, wherein said stem cell is a spermatogonial, embryonic, osteogenic, hematopoietic, granulopoietic, sympathoadrenal, mesenchymal, epidermal, neuronal, neural crest, O-2A progenitor, brain, kidney, pancreatic, liver or cardiac stem cell.

160.(New) The selectable transgenic stem cell of Claim 157, wherein said stem cell is a selectable transgenic female or a selectable transgenic male germ cell.

161.(New) A transgenic non-human mammal comprising the stem cell of Claim 157.

162.(New) A male gamete obtained by the method of Claim 149.

163.(New) Semen comprising the male gamete of Claim 162.

164.(New) A method of producing a transgenic non-human mammalian line having native germ cells, comprising

breeding the non-human mammal of Claim 161 with a member of the opposite sex of the same species; and selecting progeny for stem cell-specific expression of a xenogeneic fluorescent or light-emitting protein.

165.(New) A selectable transgenic stem cell obtained by:

obtaining a male germ cell from a non-human mammal;

transfecting said male germ cell in vitro with a transfection mixture comprising at least one transfecting agent and at least one polynucleotide comprising a transcriptional unit of a human cyclin A1 promoter sequence operatively linked to a DNA encoding a fluorescent or light-emitting protein, wherein said male germ cell is selected from the group consisting of spermatogonial stem cells, type B spermatogonia, primary spermatocytes, preleptotene spermatocytes, leptotene spermatocytes, zygotene spermatocytes, pachytene spermatocytes, secondary spermatocytes, spermatids, and spermatozoa;

causing said polynucleotide to be taken up by, and released into said male germ cell; and

fertilizing an ovum with said male germ cell such that a transgenic progeny expressing said fluorescent or light-emitting protein in at least one of its stem cells is obtained, said stem cell(s) being selectable from non-stem cells by detecting light emissions from said stem cell(s).

166.(New) The selectable transgenic stem cell of Claim 165,

wherein said stem cell is a pluripotent, multipotent, bipotent, or monopotent stem cell.

167.(New) The selectable transgenic stem cell of Claim 165, wherein said stem cell is a spermatogonial, embryonic, osteogenic, hematopoietic, granulopoietic, sympathoadrenal, mesenchymal, epidermal, neuronal, neural crest, O-2A progenitor, brain, kidney, pancreatic, liver or cardiac stem cell.

168.(New) The selectable transgenic stem cell of Claim 165, wherein said stem cell is a selectable transgenic female germ cell or a selectable transgenic male germ cell.

169.(New) A transgenic non-human mammal comprising the selectable transgenic stem cell of Claim 165.

170.(New) Semen comprising the male germ cell of Claim 168.

171.(New) A method of producing a transgenic non-human mammalian line having native germ cells, comprising

breeding the non-human mammal of Claim 169 with a member of the opposite sex of the same species; and selecting progeny for stem cell-specific expression of a xenogeneic fluorescent or light-emitting protein.

172.(New) A transgenic non-human mammalian cell containing a nucleic acid construct, said nucleic acid construct comprising a human cyclin A1 promoter having nucleotide sequence (SEQ. ID. NO.:2), or an operative fragment [or non-human mammalian homologue thereof], or an operative derivative [of any of these].

173.(New) A transgenic non-human mammal comprising the cell of Claim 172.

174.(New) The transgenic non-human mammalian cell of Claim 172, wherein said cell is a transgenic stem cell.

175.(New) The transgenic stem cell of Claim 174, wherein said stem cell is a pluripotent, multipotent, bipotent, or monopotent stem cell.

176.(New) The transgenic stem cell of Claim 174, wherein said stem cell is a spermatogonial, hematopoietic, embryonic, osteogenic, granulopoietic, sympathoadrenal, mesenchymal, epidermal, neuronal, neural crest, O-2A progenitor, brain, kidney, pancreatic, liver or cardiac stem cell.

177.(New) The transgenic stem cell of Claim 174, grown in vitro.

178.(New) The transgenic stem cell of Claim 177, grown in the presence of an inhibitor of DNA methylation.

179.(New) A transgenic non-human mammal comprising the transgenic stem cell of Claim 174.

180.(New) The transgenic non-human mammal of Claim 179, wherein said non-human mammal is a non-human primate, a mouse, a rat, a rabbit, a gerbil, a hamster, a canine, a feline, an ovine, a bovine, a swine, a pachyderm, an equine, or a marine mammal.

181.(New) A method of obtaining a selectable transgenic stem cell of a mouse, comprising:

injecting into a gonad of a male mouse a transfection mixture comprising at least one transfecting agent and at least one polynucleotide comprising a transcriptional unit of a human cyclin A1 promoter sequence operatively linked to a DNA encoding a fluorescent or light-emitting protein, wherein said gonad contains a male germ cell of the mouse, and wherein said germ cell is selected from the group consisting of spermatogonial stem cells, type B spermatogonia, primary spermatocytes, preleptotene spermatocytes, leptotene spermatocytes, zygotene spermatocytes, pachytene spermatocytes, secondary spermatocytes, spermatids, and spermatozoa;

causing said polynucleotide to be taken up by, and released into, said germ cell; and

incorporating said polynucleotide into the genome of said germ cell, whereby a selectable transgenic stem cell is obtained expressing said fluorescent or light-emitting protein, by which said stem cell can be isolated or selected from a non-stem cell.

182.(New) The method of Claim 181, further comprising, after incorporating said polynucleotide into the genome of said germ cell, breeding said male mouse with a female mouse to obtain a transgenic progeny expressing said fluorescent or light-emitting protein in at least one of its stem cells.

183.(New) The method of Claim 182, wherein breeding is by in vitro or in vivo fertilization of an ovum of said female mouse.

184.(New) The method of Claim 181, wherein said cyclin A1 promoter sequence comprises SEQ. ID. NO.:2, or an operative fragment [or non-human homologue] thereof, or an operative derivative of any of these.

193.(New) A transgenic mouse comprising the selectable transgenic stem cell of Claim 191.

194.(New) Semen of a male mouse comprising a male gamete obtained by the method of Claim 188.

195.(New) A method of producing a transgenic murine line having native germ cells, comprising

breeding of the transgenic mouse of Claim 193 with a mouse of the opposite sex; and selecting progeny for stem cell-specific expression of a xenogeneic fluorescent or light-emitting protein.--.

RSP795-8.WPD

EXHIBIT A

P07 43598 (CEDAR) PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Readhead *et al.*	Art Unit:	1633
Serial No.:	09/191,920	Examiner:	D. Clark
Filed:	November 13, 1998		

For: **TRANSFECTION, STORAGE AND TRANSFER OF MALE GERM CELLS FOR GENERATION OF TRANSGENIC SPECIES & GENETIC THERAPIES**

DECLARATION OF DR. CAROL W. READHEAD

UNDER 37 C.F.R. § 1.132

1. I, Carol W. Readhead, residing at 2185 San Pasqual Street, Pasadena, CA 91107, declare that I have personal knowledge of the facts averred herein.

2. I am a co-inventor of the invention described and claimed in U.S. Patent Application Ser. No. 09/191,920 for TRANSFECTION, STORAGE AND TRANSFER OF MALE GERM CELLS FOR GENERATION OF TRANSGENIC SPECIES & GENETIC THERAPIES, claiming priority from U.S. Provisional Application No. 60/065,825, filed on November 14, 1997. Professor Robert Winston, who resides at 11 Denman Drive, London NW 11 6RE, United Kingdom, is a co-inventor. Together Professor Winston and I are the sole inventors of the claimed invention.

3. Professor Winston and I successfully introduced exogenous genetic material into male germ cells of mice, in accordance with the claimed in vivo method of incorporating a polynucleotide into a male vertebrate's germ cells. Subsequent to natural matings of the treated animals, we further obtained production of transgenic progeny that expressed the marker in their cells. These events are described in detail in the following paragraphs 4-8b.

4. Microsurgery. After depopulation of the testis, viral particles were delivered to the seminiferous tubules as follows: Mice were anaesthetised with isofluorane (0.5-2% in oxygen). Each testis was exposed through a midline abdominal incision. Using a microsurgical approach (Winston, R.M.L., *Microsurgical reanastomosis of the rabbit oviduct and its functional and pathological sequelae*, Brit. J. Obstet. Gynaecol. 82:513 - 522 [1975]; Zeiss microscope at magnification 4 to 50x) the tissue bundle containing the vasa efferentia was visualised. Dissection from the surrounding fat was aided by a stream of phosphate buffered saline forced through a fine needle. A quartz glass micropipette was back-filled with 10 μL viral particles (10^9pfu/ml) mixed with 1 μL polybrene (80mg/mL). This was attached to a

micropipette (Eppendorf) and the particles introduced into the vas efferens under 2.2 bar pressure in pulses of 1.5 seconds, controlled by foot pedal. Earlier trials using 1% Bromophenol dye showed that most seminiferous tubules could be filled, but during treatments, no dye was used and small air bubbles were introduced into the liquid containing viral particles to confirm dispersion into the seminiferous tubules. To preserve fertility, only single vasa efferentia were injected, reducing injury to the remaining ducts.

5 Preparation of the Viral Vector. The plasmid, pHR'-CMVLacZ (Naldini, L., *et al., In vivo gene delivery and stable transduction of nondividing cells by a lentiviral vector*, Science 272: 263-267 [1996]), was modified by replacing the *Bam*HI – *Xho*I fragment containing the *LacZ* gene with a fragment containing the EGFP gene ('humanised' GFP, Clontech). For the production of viral particles 40 μg plasmid DNA was used to transfect a 10-cm plate of 293T cells. The 40 μg of plasmid DNA was made up of 10 μg pCMV R9, 20μg of modified pHR' and 10 µg envelope plasmid Vesicular-stomatitis-virus-glycoprotein (VSV-G) pseudotyped vectors were produced by contransfection of the vector plasmid with the Moloney murine leukemia virus (MLV) gag-pol packaging plasmid pCMV-GAGPOL and the VSV-G plasmid. The supernatant was harvested 48-60 hours after transfection, subjected to high speed centrifugation, filtered through 0.45 µm filters and assayed. The transducing viral particles had the MLV restricted envelope protein, *env*, substituted with a broad-spectrum *env* protein from the vesicular stomatitis virus.

6 In Vivo Transduction of Male Germ Cells. Six mice were now treated with viral particles containing the transducing vector pHR' (10^9 particles/mL). A single vas efferens was injected with a volume of 10µL retroviral concentrate together with 1µL (80mg/mL) polybrene. After 24 days the mice were sacrificed and the testes removed and fixed for cryosectioning and histological examination. Testes were fixed for 48 hours in 4% Paraformaldehyde pH 7.4, and placed in 20% sucrose in phosphate saline buffer pH 7.4 at 4°C for 24 hours. They were embedded in OCT and stored at –70°C. They were cryosectioned at 8µm and viewed in a Zeiss 410 confocal microscope (Fig. 1). Nearly all tubules sectioned contained cells expressing GFP Expression was highest in Sertoli and spermatogonia cells (Fig. 1a -b).

7. Natural Matings with Females after Transduction of Male Germ Cells. Eleven C57/B1/6J young males were then selected to test whether transduced male germ cells could transmit the retrovirally integrated transgene to the next generation. Six of these mice were treated with a bolus of busulfan (IP; 4µg/gm body wt.) 14 days before in vivo transduction microsurgery in accordance with the in vivo method of incorporating exogenous genetic material into the genome of a vertebrate, as described above, and three received the same dose only one week before in vivo transduction. Two other mice were not pre-treated with busulfan before the in vivo transduction operation. Lentiviral particles were introduced into the seminiferous tubules. After 14 weeks, B6D2F1 females were introduced into cages with the males. Transduced males fathered at least two successive litters. Litters were conceived 14, 15, 19 and 20 weeks after transduction. All the males, except one dying immediately after surgery, fathered transgenic offspring. (Table 1, next page).

Table 1. Production of transgenic offspring per litter fathered by treated males at various times after mating.

Mouse #	Pre-treatment	14 weeks	15 weeks	19 weeks	20 weeks
1	Busulfan 1 week	-	2/9 (22%)	8/10 (90%)	0/9 (0%)
2	Busulfan 1 week	-	1/7 (14%)	1/7 (14%)	2/7 (28%)
3	Busulfan 1 week	4/7 (57%)	1/8 (12%)	4/6 (66%)	0/7 (0%)
4	Busulfan 1 week	7/8 (87%)	3/7 (43%)	1/6 (17%)	1/8 (12%)
5	Busulfan 2 weeks	5/6 (83%)	8/9 (89%)	-	0/8 (0%)
6	Busulfan 2 weeks	-	2/8 (25%)	8/8 (100%)	1/9 (11%)
7*	Busulfan 2 weeks	-	-	-	-
8	Busulfan 2 weeks	-	6/6 (100%)	-	1/8 (12%)
9	Busulfan 2 weeks	-	8/8 (100%)	-	3/10 (30%)
10**	none	2/5 (40%)	5/6 (83%)	-	-
11	none	3/7 (43%)	7/8 (88%)	-	0/6 (0%)

*Mouse No. 7 died immediately after surgery.

**Mouse No. 10 died 17 weeks after surgery.

8. PCR and Southern blot analysis of DNA from embryonic offspring. Embryos at approximately embryonic day 12 5, were screened for presence of the transgene by polymerase chain reaction (PCR) and Southern blot analysis. For the PCR, GFP specific primers were used and a radiolabeled GFP cDNA probe was used for the Southern blot analysis (Fig. 2). DNA was purified from embryos using the Gentra purification system. The presence of the transgene was ascertained using PCR amplification with the following GFP specific primers:

(A) forward primer: 5'-GGT GAG CAA GGG CGA GGA GCT-3'

(B) reverse primer: 5'-TCG GGC ATG GCG GAC TTG AAG A-3'

The PCR cycling conditions were: denaturing 94°C for 1 minute, annealing at 60°C for 1 minute and extension at 72°C for 3 minutes. PCR ran for 35 cycles and yielded a specific GFP product 470 base pairs in length. Each cycle step can be reduced to one second - "one second PCR" to yield a distinct 470-bp PCR amplification product. Southern blot analysis

was also done on the same embryo DNA extracts. The DNA was cut with *Bam*HI-*Xho*I, run on a 0.8% agarose gel and blotted overnight in 20x SSC onto Hydrobond XL paper. The blots were hybridised overnight at 65°C with a ^{32}P -radiolabeled *Bam*HI-*Xho*I GFP fragment isolated from the pHR'plasmid. The blots were washed at 65°C (30 minutes) each in 2x SSC with 0.1% SDS, 1x SSC with 0.1% SDS, 0.1 X SSC with 0.1% SDS and exposed to X-ray film.

8a. PCR and Southern analysis showed that a high percentage of transgenic offspring were obtained in litters conceived within 15 weeks. The results are summarized in Table 1. By 20 weeks the percentage of transgenic progeny had dropped in all of the treatment groups, implying that the self-renewing spermatogonia were not transduced, but rather a population of differentiating spermatogonia. Once the daughter cells from this population had matured and left the testis they were not renewed (Huckins, C. & Oakberg, W.F , *Morphological and quantitative analysis of spermatogonia in mouse testes using whole mounted seminiferous tubules. II. The irradiated testes*, Anat. Rec. 192:529-42 [1978]) In Table 1, the ratios are the number of transgenic offspring out of the total number of embryos in the litter.

8b. Although pre-treatment with busulfan enhanced the transduction of spermatogonia, mice untreated with busulfan also generated transgenic offspring. Male germ cells take 60 days to differentiate from spermatogonia (Russell, L.D., *et al.* In: *Histological and Histopathological evaluation of the testis*, Cache River Press [1990]), undergo meiosis and form spermatozoa. Since conception was more than 60 days after transduction, it is presumed that the transgenic offspring were conceived from differentiated daughter cells of transduced spermatogonia. EGFP expression was driven by the CMV promoter and was evident in the testicular cells of the founder males 24 days after infection. The animals that were infected did not appear to have toxic side effects with the possible exception of one dying 17 weeks after surgery.

Carol W. Readhead 11/14/00

Carol W. Readhead Date

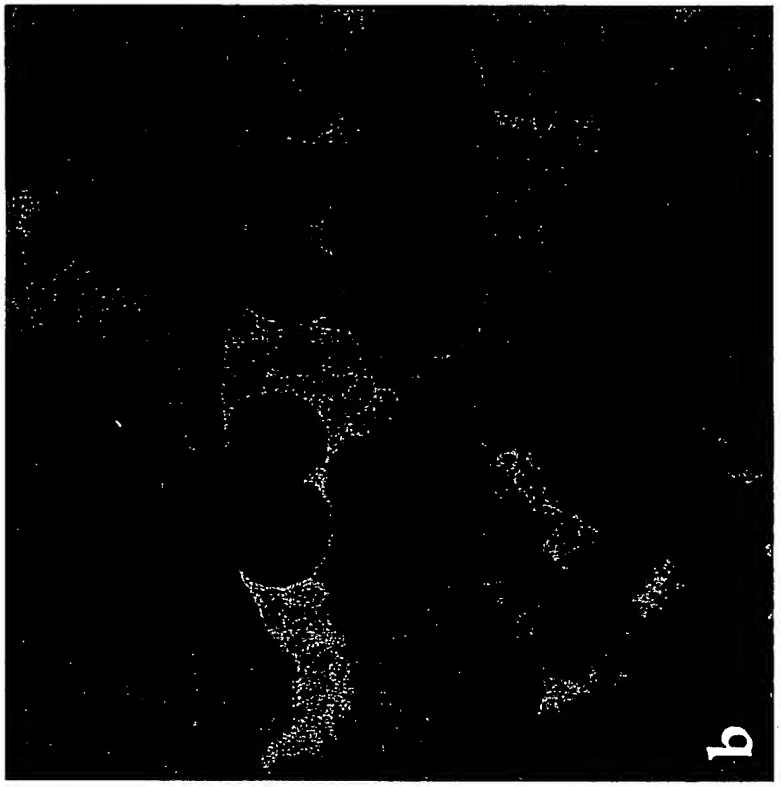
Senior Research Associate

Professor, Department of Biology

California Institute of Technology

Figure 1 shows testicular cells transduced by a pseudotyped lentiviral vector expressing Green Fluorescent Protein (GFP) in Zeiss 410 confocal images (wavelength 488 nm; 19 stacked images) of a cryosection of mouse testis. Figure 1(a) shows a transduced Sertoli cell expressing GFP. Figure 1(b) shows transduced spermatogonia; GFP expression is visible in the cytoplasm surrounding large dark nuclei.

Figure 1



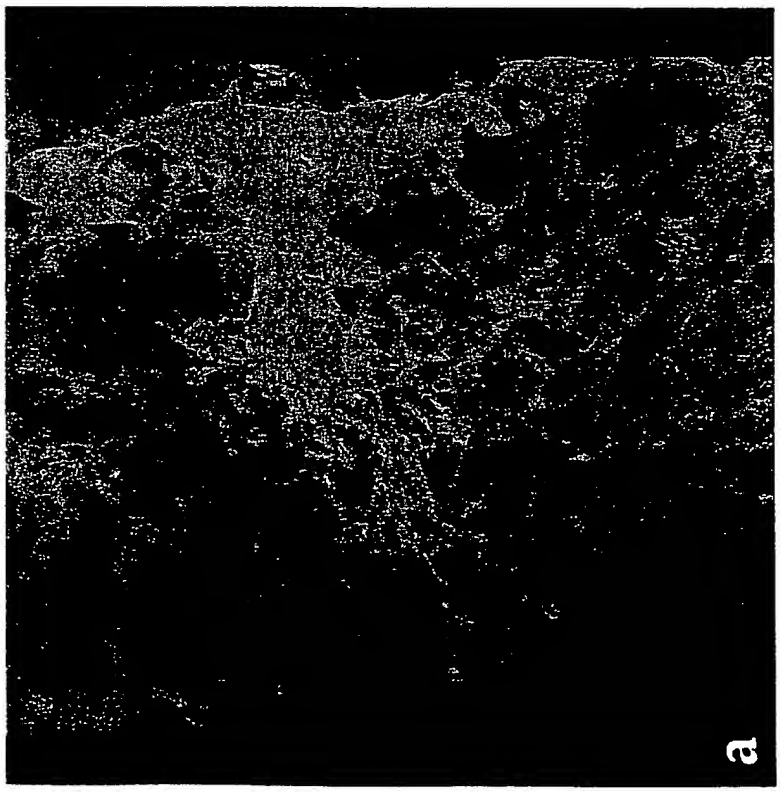


Figure 2 shows a DNA analysis from three consecutive litters of progeny from one male treated in accordance with the in vivo method of incorporating exogenous genetic material into the genome of a vertebrate. The top panel shows GFP-specific PCR amplification products separated on an agarose gel from embryonic DNA of 22 individual progeny. In this run, there was an absence of amplification from fetus No. 2, but other PCR assays confirmed the presence of the transgenic reporter gene. The bottom panel shows a Southern blot analysis of the same DNA. The Southern blot was probed with a radiolabed GFP DNA fragment.

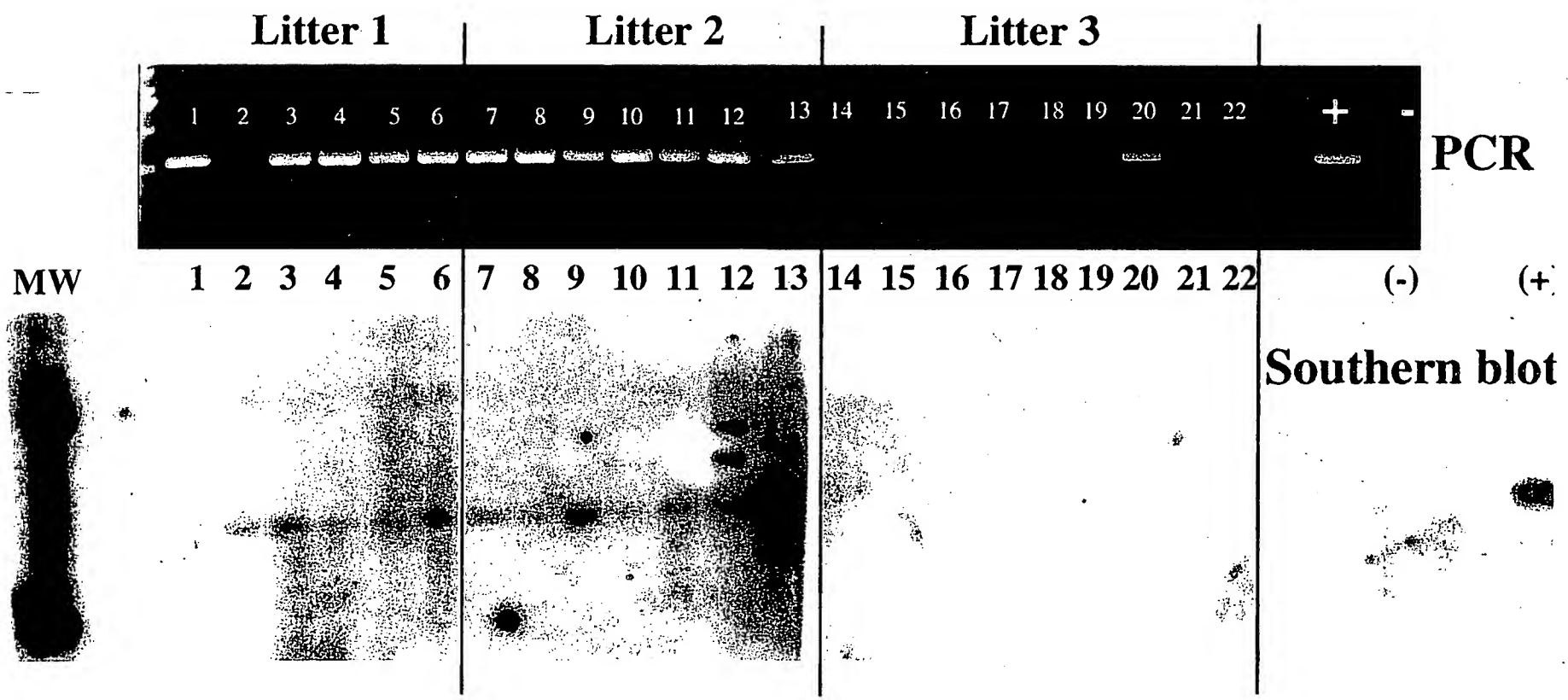

Litter 1
Litter 2
Litter 3
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 + -
PCR
MW
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 (-) (+)
Southern blot

Figure 2

EXHIBIT B

symptoms, biochemical or physiological defects, behavior, or other phenotypes of interest. The resource provides technical support for users of JAX mice to answer questions regarding genetics, husbandry, and characteristics of mutant mice. All mice can be ordered by calling The Jackson Laboratory's Customer Service Department at 1-800-422-MICE. A fee for mice is charged to partially recover strain maintenance costs and shipping expenses. For more information about the resource, contact any of the four investigators listed above. Updates on strain availability and other information are accessible by Internet at: http://www.jax.org/resources/documen ts/mmr/.

The online form for submission of strains is available at http://www.jax.org/reso urces/documents/grc/grcspontout.html.

Mice

This resource maintains strains of mice with specific mutant genes in various categories, including growth and development, reproduction, neurological, neuromuscular, vision and hearing, skeletal, immunological, skin and hair, pigmentation, kidney, and enzyme deficiencies. It also maintains stocks of mice with chromosomal aberrations including inversions, translocations, monosomy, and trisomy. In addition, several wild-derived inbred strains are maintained for linkage crosses. Details of the mouse strains available from The Jackson Laboratory are accessible by Internet at http://www.jax.org/resources/docu ments/pricelist/ or http://jaxmice.jax.org.

Index Terms

Genetic diseases, genetics, mouse, mouse models, mutations.

National Repository for Transgenic Mice and Rats

The Jackson Laboratory
600 Main Street
Bar Harbor, ME 04609-1500

Principal Investigator

Larry E. Mobraaten, Ph.D.
207-288-6373; Fax: 207-288-6149 or 6079
E-mail: lem@jax.org

Additional Contact

John Sharp, Ph.D.
207-288-6233
E-mail: jjs@jax.org

Jackson Laboratory Animal Resources
Production and Distribution Department
800-422-MICE or 207-288-5845
Fax: 207-288-6150

Research Emphasis/Objectives

The objective of this repository is to make transgenic mouse and rat models of high health quality available to investigators. Current research efforts focus on the cryopreservation of sperm and recovery of offspring from frozen and thawed spermatozoa.

Resources Provided

The National Repository for Transgenic Mice and Rats is an integral part of the Transgenic and Targeted Mutant Repository or Induced Mutant Resource (IMR) at The Jackson Laboratory. The function of the IMR is to select, import, cryopreserve, maintain, and distribute

these important strains of mice and rats to the research community. To improve their value for research the IMR also undertakes genetic development of stocks, such as transferring mutant genes or transgenes to defined genetic backgrounds and combining transgenes and/or targeted mutations to create new models for research.

Included in the repository are cancer, immunological, neurological, behavioral, cardiovascular/heart, developmental, metabolic, and other models. A list of all strains may be obtained from the IMR Web site: http://www.jax.org/resources/ documents/imr/notes.html.

In addition to serving as a repository for mouse and rat strains, the National Repository for Transgenic Mice and Rats offers to store and distribute cryopreserved embryonic stem (ES) cell lines carrying targeted mutations.

Index Terms

Cryopreservation, disease models, transgenic mice, transgenic rats.

Transgenic Mice With Altered Calcium Handling

Pharmacology and Cell Biophysics
University of Cincinnati College of Medicine
231 Bethesda Avenue, P.O. Box 670575
Cincinnati, OH 45267-0575

URL: http://blues.fd1.uc.edu/~kraniaeg/P 40_Grant1.html

Principal Investigator and Contact

Evangelia G. Kranias, Ph.D.
513-558-2327; Fax: 513-558-2269
E-mail: kraniaeg@email.uc.edu

Research Emphasis/Objectives

The recent development of phospholamban knockout and phospholamban overexpression mice has revealed that phospholamban is a major regulator of basal contractility in the mammalian cardiac, smooth, and skeletal muscles. The regulatory effects of phospholamban are mediated through the Ca^{2+}-ATPase in sarcoplasmic reticulum (SERCA2), the key enzyme involved in muscle relaxation. Dephosphorylated phospholamban is an inhibitor of the sarcoplasmic reticulum Ca^{2+}-ATPase activity, and phospholamban relieves this inhibition. The overall research hypothesis is that alterations in the levels of the sarcoplasmic reticulum Ca^{2+}-ATPase or phospholamban, and the phosphorylated states of phospholamban are associated with alterations in calcium homeostasis and function of the muscles. Thus, the long-range research goal of this resource is to generate animal models with altered expression in each of these two key Ca^{2+}-cycling proteins; and altered expression of phospholamban phosphorylation mutants in cardiac, smooth, and slow-twitch skeletal muscle. These mouse models will be made available to the biomedical community at large to carry out further in-depth studies and to elucidate the mechanisms underlying intracellular calcium regulation and physiological responses in health and disease.

Resources Provided

This resource generates and maintains mouse models with genetic alterations in either phospholamban or the sarcoplasmic reticulum Ca^{2+}-ATPase in cardiac, smooth, or slow-twitch skeletal muscle. These models are initially characterized in the resource and then they are made available to interested investigators in the scientific community. A list of all current models may be obtained from the Sarcoplasmic Reticulum Mutant Mouse Resource (SR-MMR) Web site.

Index Terms

EXHIBIT C

1: Mamm Genome 2001 Aug;12(8):575-81

Trans-NIH neuroscience initiatives on mouse phenotyping and mutagenesis.

Moldin SO, Farmer ME, Chin HR, Battey Jr JF.

Genetics Research Branch, Division of Neuroscience and Basic Behavioral Science, National Institute of Mental Health, National Institutes of Health (NIH), 6001 Executive Blvd., Room 7189, MSC 9643, Bethesda, Maryland 20892-9643, USA.

In the post-genomic era, the laboratory mouse will excel as a premier mammalian system to study normal and disordered biological processes, in part because of low cost, but largely because of the rich opportunities that exist for exploiting genetic tools and technologies in the mouse to systematically determine mammalian gene function. Many robust models of human disease may therefore be developed, and these in turn will provide critical clues to understanding gene function. The full potential of the mouse for understanding many of the neural and behavioral phenotypes of relevance to neuroscientists has yet to be realized. With the full anatomy of the mouse genome at hand, researchers for the first time will be able to move beyond traditional gene-by-gene approaches and take a global view of gene expression patterns crucial for neurobiological processes. In response to an action plan for mouse genomics developed on the basis of recommendations from the scientific community, seven institutes of the National Institutes of Health (NIH) initiated in 1999 a mouse genetics research program that specifically focused on neurobiology and complex behavior. The specific goals of these neuroscience initiatives are to develop high-throughput phenotyping assays and to initiate genome-wide mutagenesis projects to identify hundreds of mutant strains with heritable abnormalities of high relevance to neuroscientists. Assays and mutants generated in these efforts will be made widely available to the scientific community, and such resources will provide neuroscientists unprecedented opportunities to elucidate the molecular mechanisms of neural function and complex behavior. Such research tools ultimately will permit the manipulation and analysis of the mouse genome, as a means of gaining insight into the genetic bases of the mammalian nervous system and its complex disorders.

PMID: 11471049 [PubMed - in process]

EXHIBIT D

1: Curr Opin Neurol 2001 Aug;14(4):441-7

Progress in the modeling of neurodegenerative diseases in transgenic mice.

Duff K, Rao MV.

Nathan Kline Institute, New York, New York 10962, USA. Duff@nki.rfmh.org

Transgenic mouse models exist for the major neurodegenerative diseases, including Alzheimer's disease, tauopathy and amyotrophic lateral sclerosis. Although many of the mice do not completely replicate the human disease they are intended to model, they have provided insight into the mechanisms that underlie disease etiology. In the case of the Alzheimer's disease and amyotrophic lateral sclerosis models, the mice have also provided a therapeutic testing ground for the testing of agents that have been shown to have considerable clinical promise.

Publication Types:
Review
Review, tutorial

PMID: 11470959 [PubMed - indexed for MEDLINE]

EXHIBIT E

Biochem Soc Symp 2001;(67):195-202

Transgenic mouse models of Alzheimer's disease: phenotype and mechanisms of pathogenesis.

Duff K.

Nathan Kline Institute, 140 Old Orangeburg Rd, Orangeburg, NY 10962, USA.

A range of transgenic mice have been created to model Alzheimer's disease. These include mice expressing human forms of the amyloid precursor protein, the presenilins and, more recently, tau. Several of the models develop features of the disease including amyloid pathology, cholinergic deficits, neurodegeneration and cognitive impairment. Progress in the characterization and use of these model animals is discussed.

PMID: 11447835 [PubMed - in process]